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ABSTRACT

Introduction: Detecting early cardiac involvement in Fabry disease (FD) is important because therapy may alter disease progression. Cardiovascular magnetic resonance (CMR) can detect T1 lowering, representing myocardial sphingolipid storage. In many diseases, early mechanical dysfunction may be detected by abnormal global longitudinal strain (GLS). We explored the relationship of early mechanical dysfunction and sphingolipid deposition in FD.

Methods: An observational study of 221 FD and 77 healthy volunteers (HV) who underwent CMR (LV volumes, mass, native T1, GLS, late gadolinium enhancement), ECG, and blood biomarkers, as part of the prospective multicenter Fabry400 study.

Results: All FD had normal LV ejection fraction (EF 73±8%). Mean indexed LV mass (LVMi) was 89±39g/m^2 in FD and 55.6±10g/m^2 in HV. 102 (46%) FD participants had left ventricular hypertrophy (LVH). There was a negative correlation between GLS and native T1 in FD patients (r=-0.515, p<0.001). In FD patients without LVH (early disease), as native T1 reduced there was impairment in GLS (r=-0.285, p<0.002). In the total FD cohort ECG abnormalities were associated with a significant impairment in GLS compared to those without ECG abnormalities (abnormal: -16.7±3.5 vs. normal: -20.2±2.4, p<0.001).

Conclusions: GLS in FD correlates with an increase in LVMi, storage, and the presence of ECG abnormalities. In LVH-negative FD (early disease), impairment in GLS is associated with a reduction in native T1, suggesting that mechanical dysfunction occurs before evidence of sphingolipid deposition (low T1).

KEY QUESTIONS

What is already known about the subject?

Cardiac involvement in Fabry disease is characterized by progressive LVH, myocardial fibrosis and heart failure. Impairment of systolic strain measured using speckle-tracking echocardiography has previously been described in Fabry disease. CMR imaging with T1 mapping can identify cardiac involvement earlier in the disease process, however, there is only limited data investigating the relationship between myocardial strain and sphingolipid deposition.

What does this study add?

This is the first study evaluating T1 mapping and myocardial systolic strain using CMR feature-tracking in a large cohort of patients with Fabry disease. It shows that there is impairment in myocardial strain as native T1 reduces, highlighting the functional consequences of sphingolipid storage.

How might this impact on clinical practice?

This study highlights that CMR feature-tracking is a sensitive imaging biomarker that is able to identify myocardial mechanical changes in the early stages of cardiac Fabry disease.

INTRODUCTION

Fabry disease (FD) is a X-linked lysosomal storage disorder caused by mutations in the gene (*GLA*) encoding for α-galactosidase A. The progressive accumulation of complex sphingolipids, predominantly globotriaosylceramide[1] affects multiple organs, including the heart where it results in left ventricular hypertrophy (LVH), progressive cardiomyopathy, myocardial fibrosis and arrhythmias.[2] Cardiac involvement is a major contributor to morbidity and mortality in FD.[3] Evidence suggests that best outcomes may occur with early initiation of enzyme replacement therapy (ERT).[4] Early cardiac involvement is difficult to detect and the identification of early phenotypic markers is required. Change in myocardial deformation – systolic strain – also offers potential of earlier disease detection. Impairment of global longitudinal strain (GLS) has been described in FD using speckle tracking echocardiography in those with and without LVH.[5,6,7] Impaired GLS precedes any reduction in ejection fraction and is linked to worse functional status.[8]

Cardiovascular magnetic resonance (CMR) with T1 mapping has provided important insights into Fabry disease. T1 mapping is based on the magnetic resonance rate constant T1 (measured in milliseconds) that alters depending on changes in tissue characteristics – for example, fibrosis, edema and amyloid increase T1, and iron and fat decrease T1. Low native T1 values (prior to contrast administration) are postulated to indicate sphingolipid accumulation in FD, and occur in up to 59% of LVH negative FD patients.[9] CMR imaging can also quantify myocardial strain using feature-tracking (FT-CMR) but there is a paucity of knowledge regarding its application in FD. We aimed to determine whether early storage (low T1 measured using T1 mapping) would alter myocardial contractility (measured using FT-CMR) before the development of LV hypertrophy. Additionally, we aimed to evaluate if

electrical abnormalities (detected on the 12-lead ECG) alter with cardiac contractility during this earlier phase of the disease process.

METHODS

Study Population

Participants were recruited from four Fabry clinics as part of the prospective, multicenter international observational Fabry400 study (NCT03199001) – United Kingdom (UK): Royal Free Hospital London, National Hospital for Neurology and Neurosurgery London, Queen Elizabeth Hospital Birmingham; Australia: Westmead Hospital Sydney. The study was approved by the relevant Research Ethics Committees and conformed to the principles of the Helsinki Declaration. Written informed consent was obtained from all participants. Inclusion criteria for the FD cohort included: gene-positive Fabry disease and adults ≥18 years. The healthy volunteer controls (HV) were prospectively recruited and had no history of cardiovascular disease (normal health questionnaire, no cardioactive medication unless for primary prevention). Exclusion criteria included standard contraindications to CMR. All participants underwent CMR, ECG, and blood samples during the same study visit. High-sensitivity cardiac troponin T (UK) and I (Australia) (hs-TnT and hsTnI) was measured using an electrochemiluminescence-immunoassay (Roche, Basel, Switzerland; normal range 0-14ng/l and 0-15ng/l respectively).

CMR imaging

All participants underwent CMR at 1.5 Tesla (Avanto (UK), Aera (Australia); Siemens Healthcare, Erlangen, Germany) using a standard protocol including LV cines in short axis (SAX), 4-chamber, 2-chamber and 3-chamber views. Native T1 mapping was performed pre-contrast on basal and mid left ventricular SAX slices using a shortened modified Look-

Locker inversion recovery (ShMOLLI) sequence.[10] The resulting pixel by-pixel T1 color maps were displayed using a customized 12-bit lookup table, where normal myocardium was green, increasing T1 was red, and decreasing T1 was blue. Late Gadolinium enhancement (LGE) imaging was performed using phase sensitive inversion recovery (bolus administration of gadolinium 0.1 mmol/kg body weight, Gadoterate meglumine, Dotarem, Guerbet S.A., France)

CMR analysis

All images were centralized and analyzed using CVI42 software (Circle Cardiovascular Imaging Inc., Calgary, Canada). Cardiac chamber volumes and LV mass (LVM) (papillary muscles included in mass) were quantified on all subjects from a pre-contrast breath-held SAX stack of balanced steady-state free precession cine images, using previously described manual contouring methodologies.[11] Left ventricular hypertrophy (LVH) was defined as increased indexed LVM on CMR according to age and gender matched normal reference ranges.[12] Maximum wall thickness (MWT) was evaluated using semi-automated measurement on CVI42 software and a value greater than 12 mm was classified as being LVH positive.

Strain – Analysis of 2D global longitudinal strain (GLS) was obtained using CVI42, version 5.3.4. Smooth epicardial and endocardial borders were manually drawn on the end-diastolic frame of all long axis images (4-chamber, 2-chamber and 3-chamber views), and then strain (peak GLS, the most negative value during systole) was obtained from the applied automatic FT algorithm (example, Figure 1a). FT evaluates myocardial strain by utilizing a deformable 2D model and translating this onto all 2D cine slices selected over the entirety of the cardiac cycle. The extent of deformation is determined by motion of an imaginary line placed

between endo- and epicardial boundaries, which are tracked throughout the cardiac cycle by a pre-determined algorithm as previously described.[13,14] The accuracy of FT was confirmed manually for each case (by RV), and to ensure reproducibility a maximum of five operator corrections were performed.

Intra-observer reproducibility was performed by observer 1 (RV) carrying out CMR reanalysis in random subset of 30 study patients. For inter-observer variability, observer 2 (BL) independently analyzed a randomly determined subset of 20 CMR scans.

Native T1 – visual inspection of T1 color maps has shown sphingolipid deposition to be variable within the myocardium, and consequently four regions of interest (ROI) were drawn in the septal and lateral LV wall at basal and mid cavity level, taking care to avoid the blood-myocardial boundary[15] (example, Figure 1b). Since T1 is known to vary between field strength, acquisition technique and site, and gender (females typically have higher T1 than males),[15] the normal ranges of T1 values were defined as mean ± 2 standard deviations based on site-specific healthy controls from each individual center (London: males mean 956±27ms, lower limit 902ms; females mean 978±34ms, lower limit 910ms. Birmingham: males mean 947±28ms, lower limit 890ms; females mean 958±30ms, lower limit 898ms. Sydney: males mean 947±24ms, lower limit 893ms; females mean 965ms±31ms, lower limit 903ms).

ECG

Abnormal ECGs included the presence of any irregularities (prolonged or shortened PR interval, QRS duration >120ms, the presence of LVH by Cornell voltage criteria, T wave inversion in at least two contiguous leads, or the presence of ventricular ectopy).

Statistical Analysis

Statistical analyses were carried out using SPSS 22 (IBM, Armonk, NY). Continuous variables are expressed as mean ± standard deviation, categorical as frequencies or percentages. Normality was checked using the Shapiro-Wilk test. Groups were compared using the independent-samples t-test (normally distributed variables) or the Mann-Whitney U test (non-normally distributed). Chi-squared testing was utilized when comparing proportions of a variable between two groups. Troponin values were analyzed after log transformation using parametric testing. Linear regression analysis (stepwise backward method) was utilized to evaluate the relationship between multiple variables and the study outcome. Comparisons of GLS across groups of gender and T1 were assessed using an ANOVA model with post-hoc Tukey correction. A similar approach was used to assess the relationship between T1 and GLS in the LVH negative and LVH positive groups, which included three terms, namely the two factors and an interaction between them. Goodness of fit of ANOVA and regression models was assessed by visual inspection of the residuals of the model, to ensure normality.

A p-value of <0.05 was considered statistically significant. Intra- and inter-observer reproducibility was determined by calculating mean bias and 95% confidence intervals using Bland-Altman analyses and intra-class correlation coefficient (ICC) for absolute agreement.

RESULTS

Participant Characteristics

There were 298 participants in total (221 FD and 77 HV). This included (155 from London, 37 from Birmingham, and 29 from Sydney). Baseline demographics are demonstrated in Table 1. The mean FD age was 45±15 years with 85 males (38.5%) and 136 females (61.5%). The HV population was age-matched (± 2 years) with a mean age of 49.4±14 years (males 51.9%). All FD had normal LVEF (73±8.0%). Mean indexed LV mass (LVMi) was

89.0±39g/m^2 in FD and 55.6±10g/m^2 in HV. MWT was significantly higher in FD compared with HV (12±5.0mm vs. 9±1.6mm respectively, $p<0.01$). There was significant correlation between LVMi and MWT in both groups (FD: $r=0.9$ and HV: $r=0.7$, $p<0.001$). 70.8% of patients had a classical mutation and the remaining 29.2% non-classical. There were 102 (46%) FD participants with LVH.

Global Myocardial Strain

Adequate tracking quality was obtained for all study participants. Left ventricular ejection fraction (LVEF) did not correlate with LVMi ($r=0.004$, $p=0.9$). However, GLS became increasingly impaired (values becoming less negative) as LVMi increased ($r=0.728$, $p<0.001$; Figure 2a and b). GLS was impaired in the LVH-positive FD group compared to LVH-negatives and HV (table 2a, $p<0.05$). This was similar when split by sex; however the difference was greater in the male cohort (table 2a). Similar relationships were observed when correlating LVEF and GLS with MWT as a marker of myocardial hypertrophy (supplementary table 1).

Myocardial Native T1 and Strain

In the total FD cohort, 72% (n=159/221) had a low native T1 – 91% in the LVH positive subgroup (n=93/102) compared to 56% in the LVH negative subgroup (n=66/119). There was significant negative correlation between GLS and native T1 in the total FD cohort ($r=-0.515$, $p<0.05$) as shown in Figure 3a.

LVH Negative FD Population

There were 119 FD participants who were LVH negative when classified by LVMi (53.8% of total FD population). The mean age was 37±13.4 years, which was significantly lower than

the LVH positive group (53±11.7 years, $p<0.05$). 81.5% of the LVH negative cohort were female and 68.6% had a classical mutation. Mean LVMi was higher in this group than in HV (62±10.4g/m2 vs. 55.6±10.1g/m2, $p<0.05$), but maximum wall thickness (MWT) was similar (8.8±1.7mm vs. 9.0±1.6mm, $p=0.5$). GLS in LVH negative FD was better (more negative) when compared to HV (-20.3±2.9 and -19.3±2.0, $p<0.05$). When split by sex however, no significant differences were seen compared to age-matched HV (table 2a).

In the LVH-negative FD subgroup, as native T1 reduced there was also impairment in GLS ($r=-0.285$, $p<0.002$) as shown in Figure 3b. This gradient was not found to differ significantly between LVH negative and LVH positive FD (interaction term: $p=0.137$), with significant correlations between GLS and native T1 detected in both groups ($r=-0.285$ and -0.326 respectively, $p<0.002$ for both). When split by sex, LVH negative males demonstrated a greater tendency towards impairment in GLS as native T1 reduced compared to those LVH negative with normal T1 (figure 4, table 2b), however, due to a low number of males who were LVH negative with a normal T1 (n=5) this was not significant. When classifying LVH using MWT similar significant trends were observed (supplementary figure 1 and table 2).

Multivariable linear regression analysis demonstrated that LVMi and the presence of ECG abnormalities were both independent predictors of a reduction in GLS. Further regression analysis also demonstrated that LVMi and GLS were predictors of native T1. This was true in both the total population and the LVH negative cohort (supplementary table 3).

ERT

Of the total FD cohort 54.3% were on ERT and there was a significant difference in peak GLS in those taking ERT compared to those not on therapy (on ERT vs. ERT naïve: -17.6±3.8 vs. -19.7±2.9, $p<0.01$). When split by sex this difference was only present in the female population (female: on ERT -19.2±3.5 vs. ERT naïve -20.4±2.5, $p<0.05$; male: on

ERT -16.2±3.5 vs. ERT naïve -16.9±2.5, p=NS). Of the LVH negative cohort 36.1% were on ERT, however no significant differences in GLS were seen when compared to those not on ERT.

LGE

183 participants were given Gadolinium-based contrast agents (GBCA) and of these 77 (34.8%) had LGE. There was a significant difference in mean GLS between FD with and without LGE (LGE: -17.1±3.7 vs. no LGE: -19.7±2.5, $p<0.05$; LGE: -17.1±3.7 vs. HV: 19.3±2.0, $p<0.05$). In the LVH negative group, there were 14 out of 84 participants who had LGE (16.7%, all females) and there was no change in mean GLS measured (LGE: -20.4±2.2 vs. no LGE: -20.1±2.2 vs. HV: 19.3±2.0, p=0.6).

ECG

An abnormal ECG was found in 45.1% of the FD cohort. The frequency of ECG abnormalities was greater in the LVH positive cohort compared to the LVH negative group (75.2% vs. 24.7% respectively). In the total Fabry cohort ECG abnormalities were associated with a significant impairment in GLS compared to those without ECG abnormalities (abnormal: -16.7±3.5 vs. normal: -20.2±2.4, $p<0.001$). When evaluating the LVH positive cohort, this same relationship was observed in both males and females. However, in the LVH negative cohort, only females and not males had a significant difference in GLS with ECG abnormalities (table 2c).

Biomarkers

Of the FD population 156 (70.6%) had high sensitivity troponin measured (hsTnT or hsTnI), with 27.6% having an elevated level above center-specific reference ranges. Median troponin

in the total study population was 6.0 µg/L (interquartile range: 1-31 µg/L). An increasing level of troponin was associated with impairment in GLS in the total FD population ($r=0.516$, $p<0.05$). Of the LVH negative population 87 patients (73.1%) had hsTnT or hsTnI measured and only five had an elevated serum level with all others having a value <5ug/L. No significant relationship was demonstrated between strain and troponin in the LVH negative group ($r=0.169$, $p=0.118$).

Reproducibility

Intra-observer reproducibility analysis performed following repeat evaluation of 30 CMR scans by observer 1 (RV) demonstrated a mean absolute bias of 0.7±0.6 with an intra-class correlation (ICC) for single measures of 0.98 (95% CI: 0.96-0.99). Reproducibility biases were similar when assessing inter-observer reproducibility following analysis of a subset of 20 CMR scans by observer 2 (BL) – mean absolute bias 0.6±0.5 and ICC for single measures of 0.99 (95%CI: 0.97-1.0).

DISCUSSION

The main findings of this study include:

1. Impaired GLS occurs in FD in the absence of reduced LVEF. The impairment in deformation is proportionate to an increase in LVM and storage (as reflected by low T1) in the overall FD cohort, and correlates with myocardial damage as shown by both LGE and biomarker evidence of cell necrosis (troponin), and electrical abnormalities (on the ECG).

2. In LVH-negative FD (early disease), impairment in GLS is associated with a reduction in native T1, suggesting that mechanical dysfunction occurs before the onset of LVH when there is evidence of sphingolipid deposition (low T1). There is a tendency towards a lower

GLS in males with early cardiac disease, and females demonstrate no change in GLS until the onset of LVH.

3. In LVH-positive FD, impaired GLS is associated with other signs of overt cardiac involvement namely increasing LVMi and the presence of LGE.

Fabry disease affects the heart. The obvious manifestations have been ECG abnormalities, hypertrophy, and, in late stage disease, impairment and thinning.[16, 17] Biomarkers are also elevated[18, 19, 20] and valve disease can be present, but the latter is rarely a clinically significant finding. CMR has also identified LGE in early disease, which characteristically affects the basal infero-lateral wall. This was initially thought to reflect only fibrosis; however, recent developments using advanced tissue characterization with CMR parametric mapping (T1 and T2 mapping) has provided further insights. Native T1 is low in FD, representing sphingolipid accumulation [21, 22] in 85% of FD with LVH, and in up to 59% of LVH-negative patients, suggesting storage occurs early before the establishment of hypertrophy.[21, 23] When LGE is present without thinning, this has been shown to be associated with T2 elevation and hs-TnT release suggesting an inflammatory process.[19] Thus, the order and processes of phenotype development are being pieced together. ECG changes may precede echocardiographic LVH, and latest results suggest there is a pre-LVH phenotype with storage, ECG abnormalities, slight elevation of LV mass and LVEF clustering.[9]

Here, we introduce a new biomarker of myocardial mechanical dysfunction that is more sensitive than the ejection fraction to early changes in myocardial performance - GLS. This study supports the echocardiographic literature about impaired GLS in overt cardiac involvement in FD (LVH-positive disease), but offers new insights into LVH-negative disease. We have previously shown impaired GLS by speckle tracking echocardiography in a

small sample (n=25) of LVH-negative FD with low T1 compared to LVH-negative with normal T1.[21, 24] This current study expands on these findings by using a much larger cohort and is the first study to assess myocardial strain by CMR in conjunction with T1 mapping to show possible sex differences. It is also the first study to show that ECG abnormalities are associated with impaired GLS – the mechanical and electrical signals are interacting. However, further studies are required in delineate this relationship more clearly.

Sex dimorphism in the FD response to storage has been previously proposed by us in patients with overt disease.[9] That is, in addition to apparent faster storage in hemizygous males, LVH positive males appear to have reduced T1 lowering with increasing LV mass in the LVH range – suggesting the dilution of the T1 lowering sphingolipid signal by the presence of triggered sarcomeric protein. A further example, found here is that LGE can be present in LVH negative females but rarely in males.[25, 26] Here, there appears to be a trend in the way mechanical dysfunction appears also to have a sex dimorphism with female LVH-negative patients seemingly tolerating storage better than males – females tended to have preserved GLS until the presence of LVH, whereas males had impaired GLS with T1 lowering before the onset of LVH.

The limitations of this study include that it is only a single time point study with no follow-up data, but it is multicenter with a relatively large number of participants for a rare disease. A further limitation is that this study is only evaluating 2D longitudinal strain and not 3D strain. Preliminary results included assessment of 3D circumferential and radial strain, both of which demonstrated similar patterns to 2D GLS. However, when using LV short axis images to assess 3D strain parameters in FD patients with LVH and cavity obliteration, there was significant impairment in myocardial border tracking, thus excluding a large proportion of the

study cohort. Consequently, this study only assessed 2D GLS. Histological validation of T1 mapping for storage is lacking and it is likely that T1 mapping will miss the earliest storage due to the presence of a detection threshold in this technique. Further studies are also required to establish whether early institution of ERT based on a low T1 or impaired GLS in the absence of LVH affects the development of cardiac involvement.

CONCLUSIONS

In FD with LVH, myocardial strain (measured by GLS) reduces with hypertrophy, storage (measured by a low T1), ECG abnormalities and scar (measured by LGE). In early disease (LVH negative), GLS impairs as native T1 reduces.

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DISCLOSURES

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Table 1. Participant demographics and basic CMR findings

	Healthy volunteers	Fabry Total	Fabry Males	Fabry Females	p*
Sample size (n, %)	77 (100)	221 (100)	85 (38.5)	136 (61.5)	-
Age (y)	49.4±14.4	45±15.0	45±14.8	44±15.3	NS
HR (bpm)	65±9	62±12	59±12	64±12	<0.05
SBP (mmHg)	122±13	120±17	125±16	118±17	<0.05
DBP (mmHg)	76±9	73±10	74±12	72±9	NS
BSA (m^2)	1.9±0.2	1.8±0.2	1.9±0.2	1.7±0.2	<0.05
LVEF (%)	70.2±5.6	73±8.0	71±9.0	74±7.2	<0.05
LVEDV (ml)	132.1±28.4	131±31.7	146±37.3	121±22.7	<0.05
LVESV (ml)	39.6±11.9	36±17.1	43±21.0	32±12.6	<0.05
GLS	-19.3±2.0	-18.5±3.6	-16.3±3.3	-19.9±3.0	<0.05
Native T1 (ms)	955.2±29.9	879±64.3	838.8±51.5	904.6±58.4	<0.05
LGE (n, %)	0 (0)	77 (100)	36 (46.8)	41 (53.2)	<0.05
LVH-positive (n, %)	0 (0)	102 (100)	63 (61.8)	39 (38.2)	<0.05
LVMi (g/m^2)	55.6±10.1	89±39.3	116±42.6	71±24.9	<0.05
MWT (mm)	9±1.6	12±5.0	15±5.1	10±3.8	<0.05

*p-value is for Fabry male-to-female comparisons.

Table legend: HR – heart rate, SBP – systolic blood pressure, DBP – diastolic blood pressure, BMI – body mass index, LVEF – left ventricular ejection fraction, LVEDV – left ventricular end-diastolic volume, LVESV – left ventricular end-systolic volume, GLS – global longitudinal strain, LGE – late gadolinium enhancement, LVH – left ventricular hypertrophy, LVMi – indexed left ventricular mass, MWT – maximum wall thickness, NS – non-significant.

Table 2a. Mean global longitudinal strain values in various subcohorts of the total study population

	Healthy volunteers		Fabry disease		p-value
	N	Mean ± SD	N	Mean ± SD	
Study population					
Total	77	-19.3 ± 2.0	221	-18.5 ± 3.6	0.02
Male	40	-18.5 ± 1.8	85	-16.4 ± 3.3	0.001
Female	37	-20.2 ± 1.9	136	-19.9 ± 3.0	0.46
LVH positive					
Total			102	-16.4 ± 3.6	0.001
Male			63	-15.5 ± 3.4	0.001
Female			39	-17.8 ± 3.5	0.001
LVH negative					
Total			119	-20.3 ± 2.6	0.001
Male			22	-18.3 ± 1.6	0.62
Female			97	-20.9 ± 2.5	0.19

p-values are comparing Fabry to healthy volunteers in all groups.

Table 2b. Mean global longitudinal strain values in the LVH negative Fabry population classified according to native T1 compared to healthy volunteers

	Healthy volunteers		Fabry disease			
			Normal T1		Low T1	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Total	77	-19.3 ± 2.0	53	-20.5 ± 1.9	66	-20.2 ± 2.6
Male	40	-18.5 ± 1.8	5	-20.0 ± 1.0	17	-18.3 ± 1.6
Female	37	-20.2 ± 1.9	48	-20.6 ± 2.0	49	-20.9 ± 2.5
p-values from ANOVA						
Male: p=0.152			*Female: p=0.329*			

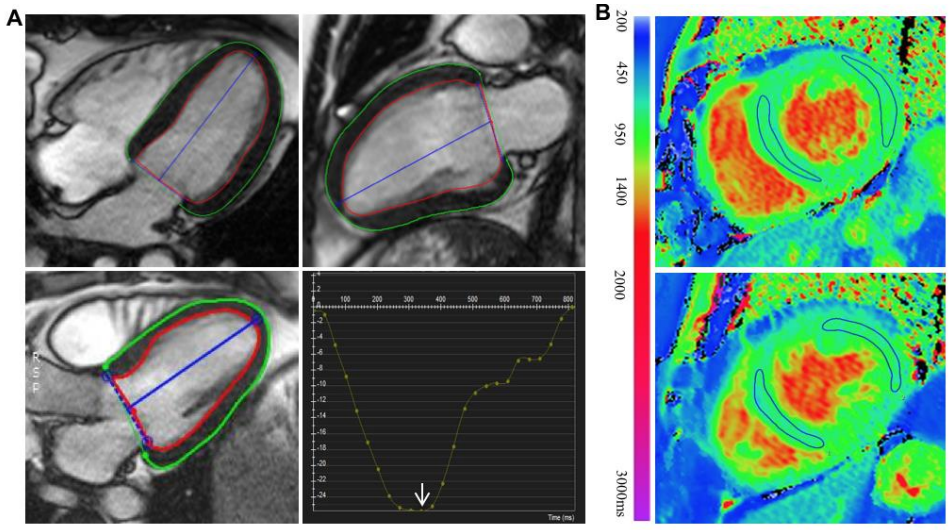
This table shows mean ± SD values for GLS. p-values are from an ANOVA model with post-hoc Tukey correction.

Table 2c. Mean global longitudinal strain values in the LVH positive and LVH negative Fabry subgroups classified according to ECG abnormalities

	N	ECG normal N	Mean ± SD	ECG abnormal N	Mean ± SD	p-value
Total cohort						
Fabry total	204	112	-20.2±2.4	92	-16.7±3.5	<0.001
Male	77	29	-18.4±2,0	48	-15.6±3.1	<0.001
Female	127	83	-20.8±2.2	44	-18.0±3.6	<0.001
LVH positive						
Fabry Total	93	23	-18.7±2.6	70	-15.9±3.4	<0.001
Male	56	13	-17.8±2.2	43	-15.2±3.1	0.006
Female	37	10	-19.9±2.7	27	-17.0±3.7	0.03
LVH negative						
Fabry Total	111	89	-20.6 ± 2.2	22	-19.3 ± 2.6	0.02
Male	21	16	-18.8 ± 1.8	5	-18.7 ± 0.5	0.80
Female	90	73	-21.0 ± 2.1	17	-19.5 ± 3.0	0.02

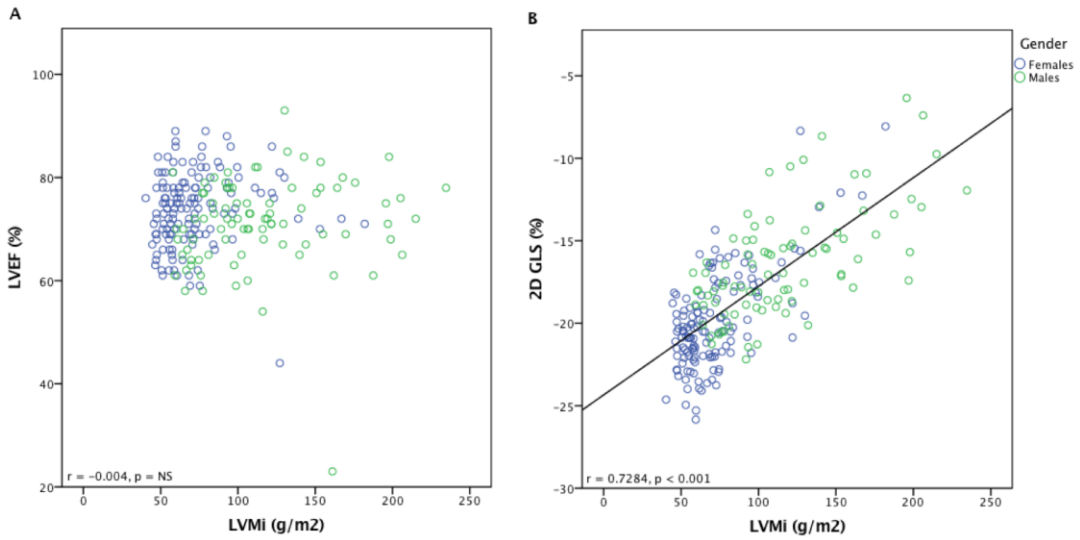
p-values are comparing ECG normal vs. ECG abnormal in all groups and are taken from an independent t-test evaluating the LVH positive and negative groups in separate t-test models.

Figure 1 Examples of cardiovascular magnetic resonance analysis techniques.



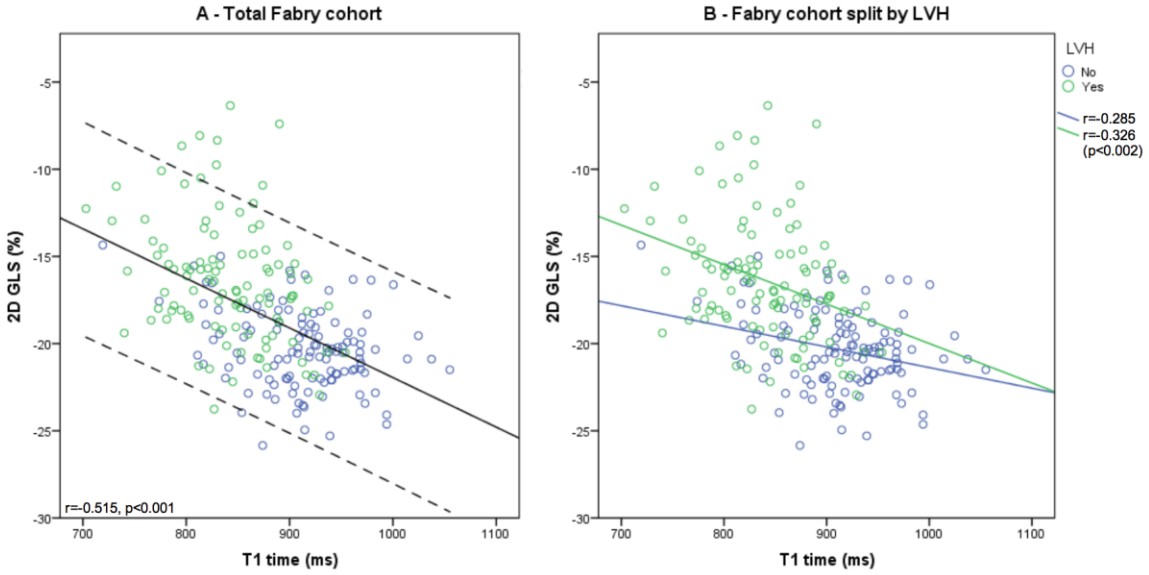
(A) Assessment of myocardial strain using feature tracking. (B) Evaluation of native T1 with regions of interest (ROI). (A) Endocardial and epicardial borders manually drawn at end-diastole on all long-axis images (four-chamber, two-chamber and three-chamber). These are used to calculate myocardial strain throughout the cardiac cycle (shown on the graph in A). Peak global longitudinal strain is the value obtained at end-systole (as shown by the arrow, –26.0% in this example). (B) Four ROIs manually drawn when evaluating T1 time. They are taken from the septum and lateral wall at basal and mid-left ventricular cavity level.

Figure 2 Scatter plots showing the relationship between indexed left ventricular (LV) mass and LV functional markers in the total Fabry cohort (males and females).



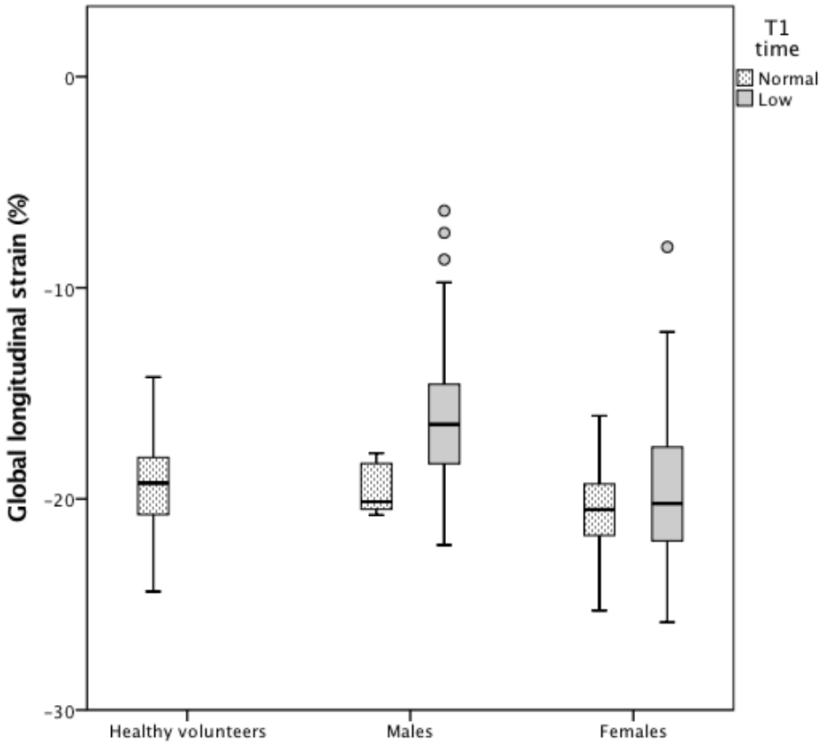
(A) No correlation between left ventricular ejection fraction (LVEF) and indexed left ventricular mass (LVMi) and (B) significant positive correlation between LVMi and global longitudinal strain (GLS), suggesting this is a more sensitive functional marker. NS - non-significant.

Figure 3 Scatter plot showing the relationship between native T1 and global longitudinal strain in Fabry disease.



An analysis of variance model found global longitudinal strain (GLS) to worsen significantly with a reduction in native T1, as shown by (A) (r=–0.515, p<0.001). The dashed lines represent 95% CIs. (B) Similar trends in the left ventricular hypertrophy (LVH)-positive (green line) and LVH-negative (blue line) groups (r=–0.326 and r=–0.285 respectively, p=0.001 for both). No significant interaction was detected between LVH and native T1 (p=0.137).

Figure 4 The relationship between global longitudinal strain and native T1 in left ventricular hypertrophy (LVH)-negative Fabry and healthy volunteers.



The graph represents the mean peak global longitudinal strain (GLS) with SD error bars. This demonstrates a trend towards an impairment in GLS in LVH-negative males with a low T1 (p=NS). NS - non-significant.